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Review

Telomere length as a biological marker in malignancy ☆

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ABSTRACT

Telomere maintenance is important for tumor cell growth and survival. Telomere length (TL) is determined by the balance between positive and negative factors impacting telomere homeostasis. In the last decade, TL has emerged as a promising clinical marker for risk and prognosis prediction in patients with malignant disorders. Tumor TL, as well as TL in healthy tissues such as peripheral blood, may carry valuable information for future treatment strategies. Here we discuss the present status of TL as a biological marker in cancer patients.

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1. Introduction

Telomere maintenance is a multifaceted process involving a large set of interacting factors, gathering at the chromosome end where the telomere is located. The main players in this process are the telomerase RNA–protein complex and the shelterin proteins, but also chromatin modifying factors are important. In the normal somatic cell, telomere shortening occurs with each cell division. It is well recognized that telomerase activity (TA), detectable in the majority of tumor cells, is the main positive power for telomere preservation and elongation. The TA level is to a large extent determined by regulation of the hTERT (telomerase reverse transcriptase) and hTR (telomerase RNA) genes. In addition, TA regulation occurs in a cell type specific manner. Furthermore, besides being activated in most malignant tumors, many normal cell types express telomerase at specific differentiation steps, often at the progenitor stage.

It is important to recognize that there is no obvious correlation between TL and TA. The actual TL is the result of all forces affecting telomere homeostasis, such as replication rate, regulatory proteins, telomerase expression, epigenetics etc. Environmental factors are also likely to have an impact on TL. As an example, oxidative stress has been shown to increase the telomere attrition rate [1]. Analyses in tumors have indicated that TA can be of value as a diagnostic and/or prognostic tool [2], but there is no consensus regarding its applicability in the clinical setting.

There is growing evidence that TL carries information that may be of clinical importance for cancer patients. It is well established that TL is important for senescence in normal cells. Malignant cells in general have shorter telomeres than their normal counterparts and there seems to be a connection between telomere shortening, genetic aberrations and risk of transformation [3]. The interest of investigating TL as a potential clinical biomarker in cancer has grown considerably in recent years. The prognostic value of TL in solid tumors was previously reviewed by Bisoffi et al. [4], and its importance in hematological malignancies has also been extensively investigated and previously reviewed [5,6].

The vast majority of studies in the field have been performed on tumor samples, but there are also investigations on blood cell TL and its possible relation to cancer risk and prognosis. It is important to discriminate between these approaches, since they focus on two completely different tissue compartments with, most likely, different telomere dynamics. When tumor DNA is studied, TL reflects the cumulative result of a variety of tumor-associated factors with impact on telomere homeostasis. TL in peripheral blood is often used as a proxy for TL in other healthy tissues. The fact that TL in peripheral blood may carry clinical information for cancer patients, as has been indicated by a growing number of studies, is intriguing. One question to be solved, however, is whether altered blood TL can contribute to disease development, or whether the TL alterations arise as a consequence of the disease. The latter case would suggest that an altered blood TL is the result of unknown mechanisms associated with the presence of a malignant tumor in the body. There is also the possibility that TL alterations detected in blood cells may cause *and* be caused by disease. In any case, previous data indicate that blood TL may serve both as a marker for cancer risk and as an independent prognostic marker for survival.

Thus, both tumor TL and TL in peripheral blood may carry information with important clinical implications. In the present review

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we give an updated summary of this research area. One important issue that is discussed is the technique used for TL measurement. It is an issue for debate and for this reason we think it appropriate to start with a description of the main methods available, including our view on their pros and cons.

2. Methods to determine telomere length

Different methods are in use for telomere length determination, all of which are based on hybridization to the telomeric repeat sequence. The traditional method, *Southern blotting*, has become established as the "golden standard" for TL determinations. In Southern blotting, restriction enzyme cut DNA is separated by electrophoresis and transferred to a membrane. Hybridization is performed with a labeled telomere-specific probe and the end-result is a smear like signal, reflecting the heterogeneous length character of the telomeres. The method is, for example, useful to evaluate telomere variability between and within different cell populations. Various algorithms can be used to assign the TL "smear" an accurate length in kilo base pairs, either as mean or peak values. However, until a common approach is used for this calculation it is still hard to directly compare TL values from different laboratories. A drawback with the technique is also that rather much DNA is needed (5–10 μg). In addition, the method is time consuming which limits its value when analyses of large series of samples are needed.

An enormous amount of clinical samples are collected in biobanks in pathology laboratories all over the world. Many of these samples are in the form of paraffin embedded tissues, including large sets of tumors of interest for retrospective analyses. DNA extracted from formalin fixed and paraffin embedded material is of poor quality and unsuitable for Southern blotting. A *slot blot methodology* has been proposed as an alternative to estimate the total telomere DNA content in such samples [7,8]. Briefly, denatured DNA is cross-linked to a filter and hybridized to a tag-labeled telomere-specific probe. A quantifiable signal is detected by using an antibody directed to the tag. The slot blot method is a promising option for analysis of paraffin embedded tissues, providing at least rough estimates of the TL. The use of PCR amplification, as described by Cawthon in 2002 [9] (see further below), could theoretically be an interesting alternative for TL determination in samples extracted from paraffin sections. In our laboratory, the PCR method have given an acceptable correlation to Southern blot data when applied on DNA from bone marrow and lymph node sections (Grabowski et al., unpublished data), but for solid tumors this approach has been unsuccessful [[10] + unpublished data from our laboratory].

Quantitative fluorescence in situ hybridization, *Q-FISH*, for quantification of telomere signals can be very informative in analyses of metaphase spreads or interphase cells [11,12]. This technique can also be used to estimate TL in paraffin sections. One advantage is that microscopically identified cells can be selected for examination. By this method, TL alterations in preneoplastic cells and early neoplasias have been identified in various tissues [13,14]. It is, however, difficult to achieve a reliable (semi)quantification of the TL, due to factors affecting e.g. the hybridization process (like tissue condition at time for fixation, fixation time, fixation efficiency, section thickness, etc.), making it hard to compare results from different laboratories. The Q-FISH technique is very useful in experimental settings but has so far not been used for prognostication in tumor patients.

Flow cytometric analysis with a fluorochrome-labeled telomere-specific probe, *flow-FISH*, is a laborious but very useful approach for TL measurements in hematopoietic cells in suspension [15,16]. The telomere content of individual cells can be obtained by this method, and in combination with DNA staining, data on e.g. telomere replication timing can be achieved [17]. Furthermore, immunophenotyping and flow-FISH can be performed simultaneously, giving the possibility to analyze specific cell types within a mixture of cells. By including an internal control cell line in each assay, the hybridization efficiency can be monitored and the control cells can be used as a standard for TL quantification [16].

A PCR based method for TL determination, *Tel-PCR*, was considered "impossible" until Cawthon [9] described his approach, taking advantage of a new primer design which minimized the risk for primer dimer appearance. The data obtained by this method correlate well with Southern blot results [9,18] and since it is PCR based, large sets of samples can be analyzed within a limited time span. In addition, the technique utilizes a small amount of DNA. Many laboratories are now using the method, but it is not uniformly accepted and the results are presented differently from laboratory to laboratory. Although the method has been applied by many researchers there is still a discussion to what extent the technique is reproducible. We believe that this, at least partly, is due to technical issues and a strict and detailed description of the method seems to be needed. We have used the Tel-PCR technique since 2005 and find it satisfactory. The inter-assay coefficient of variation for the method ranges between 4 and 8% in our laboratory, which is in the range of other reports [9,19,20]. In a very recent paper by Cawthon [21], a multiplex version of the Tel-PCR assay was presented, showing high reproducibility and very good correlation with Southern blot data. This multiplex method may well be an attractive alternative for telomere length measurements, allowing increased throughput and reduced costs.

By taking advantage of chromosome specific subtelomeric sequences Baird et al. [22] developed a PCR based technique for detection of individual telomeres, the *STELA* (single telomere length analysis) method. By this approach very short telomeres, not detected by other methods, can be visualized [22]. The method is, however, at its present stage laborious and not useful for large sets of samples or in the diagnostic setting.

In conclusion, there are several useful approaches to determine the TL and the choice depends largely on the questions asked. If single telomeres are to be analyzed the Q-FISH or STELA methods can give detailed information. TL of single cells in smears or sections can be revealed by Q-FISH, whereas flow-FISH is the method for cells in suspension. For DNA extracted from fresh cell pellets or tissue samples the options are Southern blotting and Tel-PCR and for DNA from paraffin embedded material the slot blot technique seems most promising. Warranted for the future, however, are standardized protocols (e.g. for the Tel-PCR method), so that TL values or relative TL values from different laboratories can be directly compared.

3. Telomere length as a prognostic indicator in hematological malignancies

Independent of the technique used for TL measurements there are convincing data suggesting that short telomeres in malignant hematopoietic cell populations indicate progressive disease and poor survival. A summary of published data in this field is given in Table 1 [18,23–40]. Especially for three disorders, myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL), the collected data from different research groups seem to be in good agreement. A summary of these findings are given below.

Within MDS a number of cytogenetically different subgroups are included with a varied tendency for clinical progression and development of acute leukemia. Early studies using Southern blotting indicated that short TL was associated with the frequency of genomic aberrations and to a more rapid clinical course [23,24]. The shortened telomeres in MDS have been explained as a result of increased proliferation of the malignant clone. A previous study [26], using flow-FISH, showed that CD34+ cells from MDS patients had shorter TL compared to controls and, interestingly, that the TL in CD34+ cells correlated with degree of apoptosis.

Table 1
Summary of studies investigating blood telomere length as a marker for risk and/or prognosis in hematopoietic malignancies

Tumor type	Method for TL determination	Main findings	Reference
Myelodysplasia (MDS)	Southern blot	Indications that TL reduction was linked to disease evolution and poor prognosis.	Ohyashiki et al. [23]
	Southern blot	Shortened TL associated with high frequency of cytogenetic abnormalities, high incidence of leukemic transformation and a very poor prognosis.	Ohyashiki et al. [24]
	Southern blot	TL shortening coupled to MDS subgroups and clinical progression.	Sieglova et al. [25]
	Flow-FISH	MDS granulocytes and CD34+ cells had shorter telomeres than controls. CD34+ TL correlated with CD34+ apoptosis.	Rigolin et al. [26]
Acute Myeloid Leukemia (AML)	Flow-FISH	TL shorter than in control granulocytes. Shortest telomeres found in patients with multiple genetic aberrations.	Hartmann et al. [27]
	Q-FISH	AML with multiple genetic aberrations characterized by critically short TL	Swiggers et al. [28]
	Southern blot	TL in acute promyelocytic leukemia positively correlated to PML-RARα expression. Shortened TL coupled to poorer survival.	Ghaffari et al. [29]
Chronic Myeloid Leukemia (CML)	Southern blot	Shortened TL in 56% of the patients. Patients with normal TL seemed to respond favorably to α-interferon therapy	Iwama et al. [30]
	Southern blot	Reduction in TL was associated with progression of disease	Boultwood et al. [31]
	Southern blot	Telomere reduction correlated with time to accelerated phase	Boultwood et al. [32]
	Flow-FISH	Data suggest that TL shortening can act as a marker for disease progression in CML	Brümmendorf et al. [33]
	Flow-FISH	TL reduction most pronounced in high-risk score patients at diagnosis.	Drummond et al. [34]
Chronic Lympho-cytic Leukemia (CLL)	Southern blot	TL inversely correlated to telomerase activity. Short TL and high telomerase activity associated with shorter survival.	Bechter et al. [35]
	Southern blot	Short TL associated with unmutated immunoglobulin (IGHV) genes and poor survival.	Hultdin et al. [36]
	Tel-PCR	By combining IGHV gene mutation status and TL new prognostic subgroups were indicated.	Grabowski et al. [18]
	Southern blot	Suggest that TL can be used for refined prognostication, especially for patients with unmutated IGHV genes.	Ricca et al. [37]
	Tel-PCR	Short TL associated with high risk genetic aberrations, unmutated IgHV genes, ZAP70 and CD38 expression. TL was an independent prognostic indicator for treatment free survival.	Roos et al. [38]
Myeloma	Southern blot	High telomerase activity and short TL defined a subgroup with poor prognosis. Cytogenetic aberrations correlated with telomerase activity and short TL.	Wu et al. [39]
	Southern blot	Shorter TL was associated with abnormal karyotype.	Cottliar et al. [40]

For AML the published data are sparse, but two studies [27,28] have demonstrated that AML cells have shorter telomeres compared to controls and that the shortest telomeres are found in cases with multiple genetic aberrations. For acute promyelocytic leukemia, TL was found to be positively associated with the PML-RARα fusion gene expression and short telomeres with a poor response to arsenic treatment [29].

In CML shortened telomeres is a common finding and in an early study by Iwama et al. [30], the data indicated that a small subset of CML cases with normal TL might respond favorably to interferon treatment. Later investigations from different laboratories have shown that short telomeres and increased telomere attrition rate in CML are indicative of a progressive disease [31–34].

The first study on TL in CLL indicated that TL could be a future prognostic marker, showing that short telomeres and high telomerase activity was associated with a shorter survival [35]. CLL can develop from different types of B cells depending on their "relation" to the germinal center. Hypermutation of immunoglobulin heavy chain (IGHV) genes occur in normal germinal centers (GC) as an antigen driven B cell response, and CLL can emerge from "pre-GC B cells", i.e. with unmutated IGHV genes, or from "GC B cells" (with mutated IGHV genes). Also, telomerase is activated in GC leading to telomere lengthening and therefore it was not surprising that TL in CLL was associated with IGHV gene status [18,36–38]. These studies also verified TL as a significant prognostic marker in CLL, demonstrating that short telomeres were associated with well recognized indicators of poor prognosis such as unmutated IGHV genes, high expression of CD38 and ZAP-70 and high risk genomic aberrations [18,36–38].

A similar pattern as for MDS and CLL has been indicated in myeloma for which two studies have reported that short telomeres correlated with cytogenetic aberrations [39,40]. A subgroup with poor prognosis was defined by high telomerase activity and short telomeres [39].

4. Telomere length as a prognostic indicator in solid tumors

A number of studies have reported that TL in solid tumors has potential to be used as a prognostic indicator. The research area has previously been reviewed [4] and an updated summary of published data in the field is given in Table 2 [41–59]. Several different tumor types have been investigated regarding tumor TL and clinical outcome. The majority of studies have found associations between altered tumor TL, i.e. attrition and/or elongation, and a poor outcome. The Griffith group, using slot blot assays for assessing the total telomere DNA content, has reported associations between reduced telomere content and poorer survival in both breast and prostate carcinomas [42–46]. Similarly, in sarcoma, short TL was linked to genomic instability and poor survival [59]. In contrast, long telomeres, or a high tumor to non-tumor TL ratio, have been coupled to tumors of more advanced stages and a worse prognosis in hepatocellular carcinoma [49], colorectal carcinoma [47,48], Barrett carcinoma [53], and head and neck tumors [54]. In clear cell renal cell carcinoma [55], we did not find an association between tumor TL and patient survival when comparing TL quartiles. However, there was a trend to poorer survival in patients with a high tumor to non-tumor TL ratio, and a positive correlation between TL ratio and tumor size. In lung cancer, both telomere reduction and elongation has been associated with a worse outcome [50–52]. Two studies have investigated TL as a prognostic marker in neuroblastoma [56,57]. In the most recent study [57], short TL predicted a favorable prognosis, whereas in the first study [56], short tumor TL was correlated with

Table 2
Summary of studies investigating telomere length as a prognostic indicator in solid tumors

Tumor type	Method for TL determination	Main findings	Reference
Breast	Southern blot	Telomere shortening most pronounced in Grade 3 tumors. Shorter telomeres in lymph node-negative tumors compared to lymph node-positive tumors.	Odagiri et al. [41]
	Slot blot	Reduced telomere DNA content associated with metastasis and aneuploidy.	Griffith et al. [42]
	Slot blot	Telomere DNA content correlated with stage and prognosis. Reduced telomere content found to be an independent negative prognostic factor.	Fordyce et al. [43]
	Slot blot	Telomere DNA content predicted breast cancer-free survival interval and was independent of 12 risk factors, including TNM stage and p53 status.	Heapy et al. [44]
Prostate	Slot blot	Reduced telomere DNA content associated with disease recurrence and poorer survival.	Donaldson et al. [45]
	Slot blot	Reduced telomere DNA content predicted disease recurrence independent of age at diagnosis, Gleason sum and pelvic node involvement.	Fordyce et al. [46]
Colorectal	Southern blot	TL positively correlated with tumor stage. High tumor to non-tumor TL ratio (>0.90) associated with poorer overall survival.	Gertler et Al. [47]
	Southern blot	Long TL associated with a poor outcome. Favorable outcome for telomerase-positive tumors with small tumor to non-tumor TL ratio (≤0.66) or TRF1 overexpression.	Garcia-Aranda et al. [48]
Hepatocellular	Southern blot	Advanced tumors with poor prognosis showed high telomerase activity, high mitotic instability, long telomeres and a high tumor to non-tumor TL ratio (>0.80).	Oh et al. [49]
Lung	Southern blot	Both telomere reduction and elongation observed but no significant association with prognosis shown.	Shirotani et al. [50]
	Southern blot	Alterations in tumor TL, i.e. reduction or elongation, associated with a worse outcome.	Hirashima et al. [51]
	Southern blot	Short tumor TL was an independent negative prognostic factor.	Frias et al. [52]
Oesophagus	Southern blot	Poor survival for patients with a high tumor to non-tumor TL ratio (>1.17). TL ratio found to be an independent prognostic factor.	Gertler et al. [53]
Head and neck	Southern blot	Poor disease-free survival for patients with long tumor TL.	Patel et al. [54]
Renal	Tel-PCR	No association between tumor TL and patient survival when comparing TL quartiles. A trend to better survival found for patients with a low TL ratio [≤ median (0.55)].	Svenson et al. [55]
Neuroblastoma	Southern blot	Short tumor TL correlated with advanced tumor stages, increased S-phase fractions in tumor cells, and poor survival.	Hiyama et al. [56]
	Southern blot	Long or unchanged tumor TL associated with a poor outcome.	Ohali et al. [57]
Glioblastoma multiforme	Southern blot	Tumors displaying an ALT phenotype with long telomeres had a better prognosis compared to patients with non-ALT tumors.	Hakin-Smith et al. [58]
Sarcoma	Southern blot	Short tumor TL associated with genomic instability and a worse outcome.	Avigad et al. [59]

advanced stages and poor survival. Finally, in glioblastoma multiforme, patients with tumors displaying an ALT phenotype had a more favorable outcome [58].

Hence, although not entirely consistent, most studies have indicated that tumor TL alterations are associated with a worse clinical outcome. The type of alteration linked to a poorer survival (short vs. long TL) might depend on the tumor type. At the present time, the underlying mechanisms remain unclear. As for short TL and its association to a poorer outcome, it is logical to consider the relation between short TL and genomic instability. It has previously been reported that TL abnormalities occur early in the carcinogenic process [13,14]. The association between long telomeres and a poorer clinical outcome can only be speculated upon. For the tumor types concerned, it might be that telomerase is more highly expressed in advanced tumors. Indeed, in hepatocellular carcinoma, Oh et al. [49] reported that tumors of advanced stages displayed high telomerase activity and long telomeres. It is not unreasonable to think that long telomeres in the tumor reflect telomere stabilization which might be of advantage for tumor progression.

5. Blood telomere length as a potential predictor for cancer risk and prognosis

There is a need for reliable, easily measurable biological markers for risk assessment and prognostication of malignancies. In recent years, a growing number of studies have focused on analyzing telomeres in peripheral blood cells in relation to cancer risk. Since blood is an *easily accessible* biological sample, blood TL is an interesting candidate as a biological marker. However, previous reports from this research area are not unequivocal. A summary of publications and their main findings are shown in Table 3 [19,55,60–67]. In renal [63,67], lung [63,64], bladder [63,65], head and neck [63] and oesophagus [66] carcinomas, short TL in peripheral blood have been associated with increased cancer risk. Similar data have been reported for malignant lymphoma [68]. Regarding breast cancer, a study in sister sets found a non-significant association between short TL and increased cancer risk [60]. In contrast, in newly diagnosed women with spontaneous breast tumors, cancer risk increased with increasing TL [62]. In yet another study, no significant differences in TL were found between breast cancer patients and controls, or between affected women before and after treatment [61]. In a recent study on skin cancer, long blood TL was associated with a higher risk of melanoma but a lower risk of basal-cell carcinoma, whereas no obvious association was found between TL and risk of squamous-cell carcinoma [19]. Hence, the majority of studies have found blood TL alterations in cancer patients when compared to healthy controls. It might be that the type of TL alteration differs depending on the tumor type. Differences in the methodology of measuring TL might also contribute to the discrepancy. In addition, the type of blood cells that are analyzed can vary between different studies (i.e. buffy coats vs. lymphocytes etc.). However, in the skin cancer study [19], different TL alterations were found in different tumor types, even though the method for measuring TL (real-time PCR) was the same for all tumors.

The biological mechanism(s) behind the reported association between blood TL alterations and increased cancer risk is not clear. For example, it is not evident whether the TL alterations observed in peripheral blood reflect a secondary phenomenon caused by the cancer disease, or if the alterations are in fact part of the cancer etiology; or both. Oxidative stress has been associated with tissue aging [69] as well as telomere shortening [1], and it has been proposed

Table 3
Summary of studies investigating blood telomere length as a marker for risk and/or prognosis in solid malignancies

Tumor type	Method for TL determination	Main findings	Reference
Breast	Tel-PCR	A non-significant association found between short telomeres in leukocytes and increased breast cancer risk.	Shen et al. [60]
	Southern blot	No significant differences in TL detected between breast cancer patients and controls, or between affected women before and after treatment.	Barwell et al. [61]
	Tel-PCR	Breast cancer risk increased with increasing blood TL. Long TL (>median) found to be an independent negative prognostic factor.	Svenson et al. [62]
Lung	Q-FISH and Southern blot	Short TL in lymphocytes associated with increased risk of lung cancer	Wu et al. [63]
	Tel-PCR	Lung cancer risk increased with decreasing lymphocyte TL. Strongest association between short TL and increased cancer risk found in patients with small cell carcinoma, as compared to squamous cell carcinoma and adenocarcinoma.	Jang et al. [64]
Bladder	Q-FISH and Southern blot	Bladder cancer risk increased with decreasing lymphocyte TL.	Wu et al. [63]
	Tel-PCR	Short TL associated with increased risk of bladder cancer. Differences in TL across categories of pack-years of smoking and between genders observed.	McGrath et al. [65]
Oesophagus	Tel-PCR	Short leukocyte TL associated with increased risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus, most notably among NSAID nonusers, ever smokers, and patients with low waist-to-hip ratio	Risques et al. [66]
Skin	Tel-PCR	Long TL associated with an increased risk of melanoma. In basal-cell carcinoma, cancer risk increased with decreasing TL. No clear trend between TL and risk in squamous-cell carcinoma.	Han et al. [19]
Head and neck	Q-FISH and Southern blot	Short lymphocyte TL associated with increased risk of head and neck cancer.	Wu et al. [63]
Kidney	Q-FISH and Southern blot	Short lymphocyte TL associated with increased risk of renal cell carcinoma.	Wu et al. [63]
	Q-FISH	Short telomeres in CD4+ T cells, as well as in CD8+ T cells and overall peripheral blood lymphocytes, associated with increased risk of renal cell carcinoma.	Shao et al. [67]
	Tel-PCR	A strong association found between long blood TL (4th quartile) and a poor outcome in non-metastatic patients. Blood TL found to be an independent prognostic indicator	Svenson et al. [55]

that the cumulative burden of e.g. oxidative stress through life is registered in the telomeres of leukocytes [70]. Accordingly, it has been speculated that blood TL may act as a surrogate marker of tissue dysfunctions. In 2003, Wu et al. [63] measured TL in peripheral lymphocytes and found an association between short TL and increased risk of bladder, head and neck, lung and renal cell cancers. The authors proposed telomere dysfunction as a potential predisposition factor for cancer development. Indeed, as has been mentioned previously, there is an association between telomere shortening and genetic instability, and between genetic instability and increased risk of tumor formation [3]. However, not only short TL but also long TL has been associated with increased cancer risk, and in addition, there is still the cause and effect question. It cannot be excluded that the disease itself, or responses to the disease, causes a systemic effect which directly or indirectly affects the telomere length of peripheral blood cells. The immune response to the tumor might need to be considered. For example, inflammatory components, such as cytokines and reactive oxygen species, may have an impact on leukocyte TL. A number of cytokines have shown potential to activate telomerase [71–75] whereas oxidative stress, as mentioned above, might increase telomere attrition [1]. The proliferation rate of immune cells, and hence the telomere attrition rate, may also differ depending of the state of the immune system, which in turn might be altered due to the presence of a tumor.

It was previously reported that the inter-individual TL variation was reduced in the healthy oldest old (≥85 years) [76]. The authors had hypothesized that healthy old individuals without age-related diseases, such as cancer or cardiovascular disease, would have unusually long telomeres. The study, however, showed that this was not the case. The authors speculated that a telomere length in the "normal range" may be most protective against tumor development, since long telomeres may favor escape from senescence, whereas short TL may cause genomic instability.

One problem when discussing blood TL and cancer risk is that most studies have been cross-sectional. Large longitudinal studies are needed in order to fully examine whether blood TL may act as a predictor for tumor development. We recently conducted such a study [77] by measuring TL in two blood samples collected at ~10 years interval from 959 individuals. Of these, 343 persons were diagnosed with cancer after the second blood sample. Surprisingly, no difference in blood TL was observed between cases and controls, and the telomere attrition rate was similar for the two groups. These results indicate that blood TL might not be a predictor for cancer development per se, but since the patients suffered from a variety of tumors, it is still possible that blood TL can act as a biomarker for specific tumor types, as has been suggested by the previous cross-sectional studies. An interesting finding in our longitudinal study was that the telomere attrition rate was highly correlated to the TL at baseline, meaning that individuals with the longest telomeres at base line showed the most pronounced TL shortening over time and vice versa. Aviv et al. [78] recently published similar results, showing that age-dependent TL shortening was proportional to leukocyte TL at baseline. In both studies, a subcohort of participants even showed increased TL over time. These results add to the complexity of telomere dynamics through life.

Studies investigating blood telomere length as a *prognostic* indicator in malignancies are sparse [55,62]. We previously reported that newly diagnosed women with breast cancer had longer blood telomeres than controls [62]. Moreover, women with the longest blood TL (>median) had a significantly worse prognosis compared to women with shorter telomeres. Interestingly, blood TL was found to be an independent prognostic marker. In a recent study [55], we investigated TL in peripheral blood, kidney cortex, and tumors of patients with clear cell renal cell carcinoma. Importantly, and similar to our breast cancer study, patients with the longest blood TL (4th quartile) had a significantly worse prognosis compared to patients with shorter blood TL. Again, blood TL turned out to be an independent prognostic indicator. In contrast, TL in kidney cortex or tumor tissues did not predict survival when comparing TL quartiles. The reason to why patients with long leukocyte TL showed a poorer outcome compared to patients with shorter TL remains unclear and can only be speculated upon. Nevertheless, our findings indicate that blood TL is a prognostic indicator, which could be of importance for future treatment strategies. A question to be solved, however, is how TL measurements could become valuable not only for large groups, but also for single individuals. Setting a "normal range" regarding TL is difficult, due to the large inter-individual variability in TL among individuals of the same age.

6. Concluding remarks

For proper evaluation of TL as biomarker in malignancy it is necessary to recognize the complexity of telomere maintenance and the fact that we still lack much information on this issue. The methodology used for TL measurements might have an impact but it seems as if Southern blotting, flow-FISH and Tel-PCR all give relevant information provided that proper controls are used, even though it might be difficult to directly compare data between laboratories. Regarding TL abnormalities in malignant cells and clinical outcome the picture is multifaceted. For hematogical malignancies the published data are in good agreement for MDS, CML and CLL, showing that short telomeres indicate progressive disease and a poor outcome. For solid tumors the data are more heterogeneous. A possible prognostic impact of TL abnormalities seems to be tumor type dependent and more data on well characterized tumor materials are required. It is evident that the appearance of a malignancy affects the whole body, and it is possible that the alterations in blood TL seen at diagnosis reflect responses to the tumor. Similar as for solid tumors, the reported TL alterations in blood seem to differ between tumor types. Of most interest is perhaps the fact that blood TL appear to constitute a significant prognostic indicator, but additional studies on different patient cohorts are needed in order to further substantiate this indication. Large longitudinal studies and improved standardized protocols are needed for the future, to fully be able to evaluate TL as a marker in malignancies.

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